L17: Entry 8 of 14 File: USPT Mar 30, 1999

DOCUMENT-IDENTIFIER: US 5888733 A
TITLE: In situ hybridization to detect specific nucleic acid sequences in eucaryotic samples

Brief Summary Paragraph Right (61):
In the present context, the term "label" refers to a substituent which is useful for detection of hybrids formed between a binding partner and a nucleic acid. In accordance with the present invention, suitable labels comprise fluorophores, biotin, dinitro benzoic acid, digoxigenin, radioisotope labels, peptide or enzyme labels, chemiluminiscence labels, hapten, antigen or antibody labels. Examples of particular interesting labels are biotin, fluorescent labels, such as fluorescein labels, e.g. 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid and fluorescein isothiocyanate, peptide labels, dinitro benzoic acid, rhodamine, tetramethylrhodamine, cyanine dyes such as Cy2, Cy3 and Cy5, coumarin, R-phycoerythrin, allophycoerythrin, Texas Red and Princeston Red as well as conjugates of R-phycoerythrin and, e.g. Cy5 or Texas Red.

Brief Summary Paragraph Right (90):
Infection with papillomavirus has traditionally been diagnosed using histological staining, electron microscopy or immunohistochemistry designed for the detection of viral antigens. However, these methods are all relatively insensitive. Several different HPV types are known, e.g. HPV types 6, 11, 16, 18, 30, 31, 33, 35, 45, 51, and 52. From these, specific sequences may be selected as the target of detection. By way of example, an in situ hybridization protocol is described for the detection of HPV 16 infected cervical tissue using type specific binding partners.

Brief Summary Paragraph Right (94):
For detecting HPV 16, binding partners comprising polymerized moieties of formula (I)-(VII) can be used. Selection of nucleobases which will be specific for the detection of the HPV 16 is based on public available sequence information retrieved from different databases. One of the most varying regions between the different HPV subtypes are the E6/E7 open reading frame that encodes for two proteins (E6 and E7) involved in the transformation of infected cells. Appropriate binding partners, capable of forming sufficiently stable hybrids with mRNA encoding these proteins, are selected and synthesized.

Brief Summary Paragraph Right (112):
The detection of hybrids formed can be performed as described above for detection of HPV in tissue sections (see step (4) of the description of embodiment A).

Brief Summary Paragraph Left (8):
A: Procedure for performing in situ hybridization on tissue sections for the detecting of human papillomavirus (HPV)

CLAIMS:

18. A method according to claim 17, wherein the label is selected from the group consisting of fluorescent labels, biotin, digoxigenin, dinitro benzoic acid, peptide labels, rhodamine, R-phycoerythrine and cyanine dyes.

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L11: Entry 2 of 4 File: USPT Jun 17, 1997

DOCUMENT-IDENTIFIER: US 5639871 A
TITLE: Detection of human papillomavirus by the polymerase chain reaction

Abstract Paragraph Left (1):
The presence of human papillomavirus (HPV) in a sample can be detected and the HPV typed by a method that involves the amplification of HPV DNA sequences by the polymerase chain reaction (PCR). The primers used in the method are consensus primers that can be used to amplify a particular region of the genome of any HPV. The presence of HPV in a sample is indicated by the formation of amplified DNA. The HPV nucleic acid is detected by consensus probes that may be short oligonucleotide probes or long generic probes. The HPV is typed by the use of type-specific DNA probes specific for the amplified region of DNA.

Brief Summary Paragraph Right (52):
Many methods for labeling nucleic acids, whether probe or target, are known in the art and are suitable for purposes of the present invention. Suitable labels may provide signals detectable by fluorescence, radioactivity, colorimetry, X-ray diffraction or absorption, magnetism, enzymatic activity, and the like. Suitable labels include fluoruphores, chromophores, radioactive isotopes (particularly .sup.32 P and .sup.125 I), electrondense reagents, enzymes and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horse-radish-peroxidase (HRP) can be detected by its ability to convert diaminobenzidine to a blue pigment. A preferred method for HRP based detection uses tetramethyl-benzidine (TMB) as described in Clin. Chem. 33:1368 (1987). An alternative detection system is the Enhanced Chemiluminescent (ECL) detection kit commercially available from Amersham, Arlington Heights, Ill. The kit is used in accordance with the manufacturer's directions.

Brief Summary Paragraph Right (53):
Probes may be labeled with radioactive phosphorous .sup.32 P, by treating the probes with polynucleotide kinase in the presence of radiolabeled ATP. However, for commercial purposes non-radioactive labeling systems may be preferred, such as, horseradish peroxidase-avidin-biotin or alkaline phosphatase detection systems. HRP can be used in a number of ways. For example, if the primer or one or more of the dNTPs utilized in a PCR amplification has been labeled (for instance, the biotinylated dUTP derivatives described by Lo et al., 1988, Nuc. Acids Res. 16:8719) instead of the probe, then hybridization can be detected by assay for the presence of labeled PCR product. In a preferred embodiment, probes are biotinylated and detected with the ECL system described above. For example, biotinylated probes were prepared by direct biotinylation of the oligonucleotide rather than incorporation of biotin-dUTP during PCR. For 5' biotinylation of oligonucleotides direct solid phrase synthesis using biotin containing phosphoramidites was done according to Alves et al., 1989, Tetra. Let 30:3098; Cocuzza, 1989, Tetra Let. 30:6287; and Barabino et al., 1989, EMBO J. 8:4171. Solid phase synthesis of biotinylated oligonucleotides at any internal or terminal (5' or 3') position is also suitable for preparing biotinylated primers and probes (Pieles et al., 1989, NAR. 18:4355, and Misiura et. al., 1989, NAR 18:4345).

Detailed Description Paragraph Right (14):
To determine if amplification had occurred the following protocol was used. About 2 .mu.l of each reaction mixture were added to 100 .mu.l of denaturing solution (0.4M NaOH and 25 mM EDTA) for each replicate dot and spotted onto replicate, positively-charged, nylon membranes (such as Genetran 45, Biodyne B Membrane [Pall], or Biotran from ICN) using a dot-blot or slot blot apparatus. The resulting dot was rinsed once with 200 .mu.l of 20.times.SSC. The membrane was then removed from the blotter, air-dried, and exposed to ultraviolet light (with the DNA facing the light)

to covalently attach the DNA to the membrane by using a commercial UV crosslinking apparatus (50 mjoules). Amplified L1 fragments from HPV types 6, 11, 16, 18, 31, 33, 35, 39, and 45 were spotted onto each membrane as positive controls to assess cross-hybridization and optimize exposure times.

Detailed Description Paragraph Right (39):
An HPV typing assay incorporating detection by probe hybridization in a dotblot format is described in the protocol below. In the dot blot format, a small portion of the amplified DNA is denatured, applied to nylon membranes, and immobilized. Each membrane is then immersed in a probe solution to allow hybridization to the labeled probe. Replicate membranes are hybridized with either probes for one HPV type or a generic HPV probe. Probes can be radioactively labeled, as described above, or covalently conjugated to horseradish peroxidase (HRP) to provide a means of nonisotopic detection in the presence of a chromogenic or chemiluminescent substrate.

Detailed Description Paragraph Right (50):
The amplification product is analyzed by hybridization to probes essentially as described in Example 1. Biodyne B nylon membranes (ICN, Irvine, Calif.) are pretreated by incubating for at least 30 minutes in 0.1.times. SSPE and 0.5% SDS at 65.degree. C. About 3 .mu.l of each amplification reaction mixture is added to 100 .mu.l of denaturing solution (0.4N NaOH and 25 mM EDTA) and spotted onto a membrane. The resulting dot is rinsed with 400 .mu.l of 20.times. SSC. Amplified L1 fragments from HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 68, and clinical samples P88, P155 ,P238A, P291, and W13B are spotted onto each membrane as positive controls to assess cross-hybridization and optimize exposure times. Duplicate membranes are created for hybridization with probes for each of the HPV types described below and the generic HPV probe.

Detailed Description Paragraph Right (56):
In one embodiment, the amplification primers are biotinylated, as described in Levenson and Chang, 1989, supra, so that any amplified DNA that hybridizes to the membrane bound probes can be easily detected. Detection is carried out by reacting streptavidin conjugated horseradish peroxidase (SA-HRP) with the amplified DNA hybridized to the fixed probe. The HRP thus becomes bound through the SA-biotin interaction to the amplified DNA and can be used to generate a signal by a variety of well known means, such as the generation of a colored compound, e.g., by the oxidation of tetramethylbenzidine (see U.S. Pat. No. 4,789,630, incorporated herein by reference). Probes are immobilized on a nylon membrane as described in Saiki et al., 1989, supra.

Detailed Description Paragraph Right (68):
Avidin-HRP conjugate is prepared as follows. A diluent is prepared that contains 0.1 molar; 0.25% emulsion 25 (DKS International, Inc., Tokyo, Japan); 1.0% Kathon CG (Rohm and Haas, Philadelphia, Pa.); 0.1% phenol; 1.0% bovine gamma globulin. The pH of the diluent solution is adjusted to 7.3 with concentrated HCl. To this diluent, 10 nM of conjugated avidin (Vector Labs, Burlingame, Calif.) is added. One hundred .mu.l of avidin-HRP conjugate is added to each well in the plate being tested. The plate is then covered and incubated 15 minutes at 37.degree. C. and again washed as described above.

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L17: Entry 12 of 14 File: USPT May 13, 1997

DOCUMENT-IDENTIFIER: US 5629147 A
TITLE: Enriching and identifying fetal cells in maternal blood for in situ hybridization

Detailed Description Paragraph Right (63):
Preferably, the analogue should be able to permeate a cell or virus. In the case of analogues that are aurin derivatives (rosolic acid derivatives), it is preferred that the analogues have, in addition to ATA, a polar functional group such as a --CO.sub.2, --NH.sub.2, --OH, or --SO.sub.3 group, on an aromatic group; examples are chromoxane cyanine R and Chrome Azurol S. A subgroup of preferred analogues are those that block the NH.sub.2 groups on lysines.

Detailed Description Paragraph Right (186):
A negative control probe, sequences for human papillomavirus (HPV) type 16 and HPV type 18 (Table 12) were obtained from the published sequences and were accessed via the Genetic Sequence Data Bank, GenBank, version 69.0.

Detailed Description Paragraph Right (187):
Twenty separate HPV probes (10 for HPV type 16 and 10 for type HPV 18) and 180 HIV probes are synthesized by cutting the HIV sequences into several 39-base oligonucleotides and synthesized as phosphorothioate oligonucleotides using DNA synthesizers (Applied Biosystems DNA Synthesizer, Model 380B) and using the recommended A.B.I. reagents. The phosphorothioate oligonucleotides are then coupled to FITC and purified by column chromatography and HPLC.

Detailed Description Paragraph Type 1 (7):
(1) Four hundred sixteen (416) separate probes (208 for type 16 and 208 for type 18) each designed as 30-bases in length, are synthesized. However, in addition to making probes corresponding to those 416 separate oligonucleotides that together comprise probes for one strand of each of the two HPV targets, 416 additional oligonucleotide probes were made for the second strand of each of the two HPV targets. The probes for the first strand are made staggered relative to the second strand probes as regards how they map on a map of the HPV genome. As a result, one-half (15 nucleotides) of each first strand probe will be complementary (in nucleotide sequence) to one-half of one second strand probe and the other half (15 nucleotides) of that first strand probe will be complementary to a portion of another second strand probe. Staggering of the probes means that, because of the shortness of the overlap (15 nucleotides), probes of the first strand will not hybridize significantly to probes of the second strand. On the other hand, about twice as much hybridization is detected as compared to the situation where only probes corresponding to one strand are used.

Detailed Description Paragraph Table (2):
TABLE 2 Dye abbreviations Dye Number Actual Dye Name Abbreviation 12 Naphthol Blue Black Naphthol Bl. Blk. 13 Palatine Fast Black WAN Palatine F-B WAN 20 Sulforhodamine 101 hydrate Sulforhodamine 101 [CAS # 60311-02-6] Texas Red Sulforhodamine 101 acid chloride [CAS # 82354-19-6] Direct Blue Evans Blue -- 53 [CAS # 314-13-6] Fluorescein FITC isothiocyanate Hoechst 2'-[4-hydroxyphenyl]-5-[4- 33258 methyl-1-piperazinyl]-2-5'-bi- 1H-benzimidazole trihydro- chloride [CAS # 23491-45-4] Natural Hematoxylin -- Black 1 [CAS # 517-28-2] Acid Red 91 Eosin B [CAS # 548-24-3] -- Sigma 840-10 Nitroblue Tetrazolium NBT PE Phycoerythrin Cy3 FluoroLink Cy3 Fluorescent Dye, a fluorescent cyanine dye (Biological Detection Systems, Inc., 955 William Pitt Way, Pittsburgh, PA) Cy5 FluoroLink Cy5 Fluorescent Dye, a fluorescent cyanine dye (Biological Detection Systems)

Detailed Description Paragraph Table (12):

TABLE 12	Probe Designation	GenBank Locus Name
Fluorescent Label		
HPV 16	PPH16	Fluorescein
HPV 18	PPH18	Fluorescein
HIV	HUMBH102	Fluorescein

WEST Search History

DATE: Wednesday, April 03, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=ADJ		
L17	hpv and cyanine	14	L17
L16	L15 and cyanine	0	L16
L15	6268147.pn. and chip	1	L15
L14	6268147.pn. and nylon	0	L14
L13	6268147 and glass	1	L13
L12	L11 and glass	0	L12
L11	L10 and nylon	4	L11
L10	L8 and fluorescen$	8	L10
L9	L8 and cyanine	0	L9
L8	L5 and (avidin and (phosphatase or alkalinephosphatase))	9	L8
L7	L6 and nylon	2	L7
L6	L5 and chip	4	L6
L5	l1 or (papilloma).ab.	150	L5
L4	l1 or papilloma$	4841	L4
L3	L2 and nylon	1	L3
L2	L1 and (chip)	1	L2
L1	hpv.ab.	92	L1

END OF SEARCH HISTORY